Genetic Mosaicism

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FOREWORD

Many people find pleasure in looking at colorful spot-patterns on leaves and flowers, at the black/red/white coats of calico cats, or at blue spots in the eyes of brown-eyed friends. Where do mosaic spots like these come from? Are there genetic bases for mosaicism? What is their significance? This book answers these questions, presents examples for the different types of mosaics, and explains their importance in life.

I first studied genetic mosaics during the early 1970s, when we generated Drosophila female/male mosaics (gynandromorphs) and induced mitotic recombination to produce genetically labeled mosaic spots in order to better understand gene functions. Since then, the induction and the use of the different types of genetic mosaics have been important companions in my scientific life. As I approached retirement, I decided to categorize genetic mosaicism. The motivation was rather simple: to share the joy that mosaics brought me with people who, in addition to enjoying the appearance of mosaic organisms, would like to understand their significance and the underlying mechanisms of their origin. This book describes the genetic events that cause the different types of genetic mosaicism, presents examples of them, and describes methods that make good use of genetic mosaics to study environmental mutagens.

I had many motivations to put together *Genetic Mosaicism*. My grandmother, a wonderful and loving woman, had pretty black/red/white calico cats that gave births to far too many kittens to give away. She kept asking: why are all calico cats females? It was a delight to learn the explanation some years later and tell her the link between calico coloration and femaleness. The following books also inspired my study of mosaics: *Genetic Mosaics and Other Essays* (Curt Stern, 1968), *Genetic Mosaics and Cell Differentiation* (editor: Walter Gehring, 1978), *Genetic Mosaics and Chimeras in Mammals* (editor: Liane B. Russel, Plenum Press, 1978), *Chimeras in Developmental Biology* (editors: Nicole Le Douarin and Anne McLaren, 1984), and *Mosaicism in Human Skin* (Rudolf Happle, 1993). Papers by Barbara McClintock, Mary F. Lyon, Yoshiki Hotta and Seymour Benzer, Antonio Garcia-Bellido, Peter Bryant, Wilfried Janning, Trudi Schupbach, Eric Wieschaus, Karl Illmensee, Beatrice Mintz and many, many others also

greatly impacted my scientific work. Information about genetic mosaics made it possible for our research groups to elaborate ways to detect and measure the mutagenic activities of environmental agents. Of the lectures I presented over the decades, perhaps those on genetic mosaicism attracted the most attention from students. It is my sincere hope that learning about genetic mosaicism will bring pleasure to readers, as it did to the author of this book.

INTRODUCTION

Mitosis in a nutshell

The life of most multicellular organisms starts with the fusion of a female and a male germ cell. The cell they form, the zygote, is diploid: it carries a maternally and a paternally derived set of chromosomes. Two of these chromosomes - the so-called homologous chromosomes - are of identical size and structure and carry genes for the same traits in the same positions. The genetic content is identical in homozygotes and different in heterozygotes, which carry two different distinguishable varieties of the same gene, called alleles.

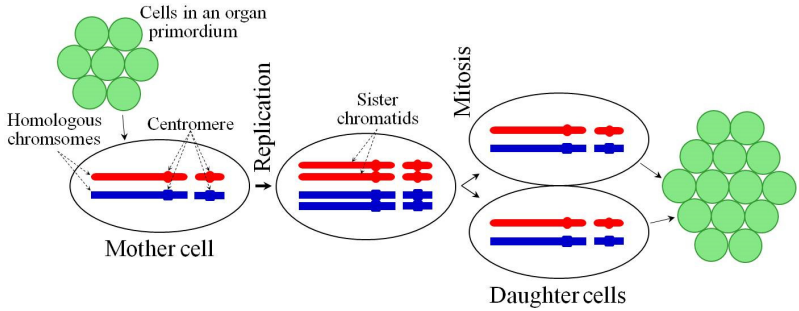

Figure 1. Schematic representation of mitosis.
Two daughter cells form from a mother cell during mitosis. The daughter cells are expected to be genetically identical to the mother cell. The red and the blue bars symbolize the maternally and the paternally derived chromosomes.

Prior to mitosis the DNA molecule replicates, i.e., two DNA molecules form from one DNA molecule. The newly formed DNA molecules are usually identical to the original, and they form two sister chromatids while they become packaged by protein and RNA molecules (Figure 1). The sister chromatids are initially attached at their centromeres, become separated during mitosis, and end up as chromosomes in the daughter cells: one in each of the daughter cells. The zygote is thus the source of all the cells that form during the subsequent mitoses and make up the

organism. One might assume that the newly forming DNA molecules, just like the daughter cells, are genetically identical to the original DNA and the zygote: they carry the same amount of DNA, the same genetic material, and the same number of chromosomes.

Genetic mosaics and their classification

Table 1. A classification of genetic mosaics

Founder germ cells	Type of genetic mosaic		Source of mosaicism
2	True genetic mosaics	Qualitative mosaics	Point mutation Mitotic recombination
		Quantitative (chromosomal) mosaics	Chromosome loss Chromosome "gain"
	Functional mosaics		X chromosome inactivation
			Position-effect variegation
			Inactivating gene effect: piebaldism and heterochromia
			RNS-interference
≥ 2	Chimeras		Delayed and/or double fertilization
			Fusion or aggregation of embryos
			Injection of embryos or cells into blastocysts
			Cell exchange

Although given this process we might expect these copies to always be perfect, that is not the case, because neither DNA replication nor mitosis is 100% error-proof. There are different types of events that may occur both during replication and mitosis that lead to the formation of daughter cells with genetic contents different from both the mother and the neighboring cells. The result is a genetic mosaic: an organism that contains cells with different genetic contents. A mosaic spot (a clone) forms when the newly formed cell and its descendants with altered genetic contents survive and stay together. When the genetic change is associated with visible features, the mosaic spot is visible. It may also happen that, although the genetic contents of the cells are identical, some genes are expressed differently in

some cells of an organ, and thus what is called functional mosaicism emerges. There are also plenty of examples of organisms with cells that originate from different sources. Such creatures are called chimeras. Most of them originate from delayed and/or multiple fertilization or through the fusion of embryos. Genetic mosaics are classified according to the mechanisms of their origins, as summarized by the table above.

1. True Genetic Mosaics

This chapter describes organisms that, despite developing from single zygotes, have bodies composed of cells with different genotypes. There are three sources of differences in the genotypes of their cells: point mutations, mitotic recombinations, and changes in chromosome number.

1.1. Point mutations and mosaic spots

Point mutations lead to the formation of mosaic spots. Examples include the green/yellow mosaic patterns in several plant species, port-wine stains, and tumours (e.g., retinoblastomas and colon and breast cancers). This chapter also summarizes how and what types of mutations bring about café au lait dark spots on the skin or leukaemia. A method based on the use of mosaic spots is presented that can be used to detect and measure the potential of environmental agents to induce point mutations.

1.2. Mitotic recombination and mosaic spots

This chapter describes mitotic recombination and its role in tumour formation as well as the origin of the different types of skin mosaicism. The chapter summarizes what we learned, by using mitotic recombination and genetic labelling of cells, about gene mapping, regeneration, the mechanism of stem cell division, and the genetic requirements of different cell types. The principles and the use of mitotic recombination to detect and quantify the potential of environmental agents to break chromosomes are also summarized.

1.3. Chromosomal mosaicism

This chapter deals with the mechanisms that ensure chromosome stability over the successive rounds of cell divisions and the dangers that stem from alterations to the diploid condition either by the loss or “gain” of chromosomes. The formation and features of what are called monosomy

and the trisomy mosaics (e.g., Turner-, Klinefelter- and Down-mosaics) are described and explained. A genetic assay to detect and measure the effects of environmental agents that induce the loss and/or gain of chromosomes is also presented.

2. Functional Mosaicism

This chapter lists the types of mosaics which, despite their cells having identical genotypes, have groups of cells in an organ that possess different phenotypes. This phenomenon is known as functional mosaicism. In functional mosaics the normal and the mutant phenotypes appear side-by-side. This phenomenon is called variegation. The sources of functional mosaicism are X-chromosome inactivation, position-effect variegation, inactivating gene action, and RNA-interference.

2.1. X chromosome inactivation

Examples of X chromosome inactivation include black/red mosaic cats and women with patches of skin with and without sweat glands. Besides describing the underlying molecular mechanism for this, the chapter briefly discusses epigenesis.

2.2. Position-effect variegation

Some chromosome rearrangements juxtapose blocks of heterochromatin with euchromatin. It can occur that in a cell of the eye primordium of a fruit fly, the heterochromatin spreads over the *white* gene and turns it off. The progeny of such a cell forms a colourless sector in the eye (which appears white). In another cell the heterochromatin does not reach the *white* gene, leaving it functional. The progeny of such a cell forms a red clone in the developing eye. As a result, a white/red, salt-and-pepper mosaicism emerges. This allows the identification of chromatin genes, which regulate chromatin compactness.

2.3. Inactivating gene effect: piebaldism and heterochromia

Melanocytes are the sources of melanin, the black, brown, and orange pigments of - among others - skin, fur, and the iris. Their founder cells migrate during embryogenesis from the neural crest to their sites of destination, where they produce pigment. The failure of neural crest cells to adequately proliferate or migrate leads to the absence of some

melanocytes and the formation of white spots in the midst of colored areas, a phenomenon called piebaldism. The functioning of melanocytes in one eye and not in the other (or more frequently in a different part of the same eye) brings about heterochromia: one eye is brown and the other blue, or part of an eye is brown and the other part blue. This chapter describes the genes involved in piebaldism and heterochromia. It also touches upon vitiligo, an acquired type of light/dark skin mosaicism seemingly without a genetic background.

2.4. RNA-interference and mosaicism

This chapter takes a look inside cells, describes the phenomenon of RNA-interference, and explains how mRNA molecules are eliminated in some cells, leading to a lack of gene function and pigments, while the same mRNA molecules function properly in other cells to form pigments.

3. Chimeras

Chimeras are organisms with cells that originate from different sources. In this chapter chimeras are discussed according to the mechanisms of their origin.

3.1. Delayed and/or double fertilization

Delayed and/or double fertilization can lead to the formation of organisms with both diploid and haploid cells. Particular interest is paid to gynandromorphs and hermaphrodites, which have both female and male cells in their bodies.

3.2. Fusion, aggregation and injection chimeras

Fusion chimeras emerge following the spontaneous fusion of embryos. Exceptional cases of female/female chimeras have attracted much attention. In cases when such women gave birth, standard DNA tests reported that they were not the mothers of their own children. Detailed analysis revealed that their children originated from a cell type in their body different from the ones that had been collected for DNA analysis. The chapter also covers female/male fusion chimeras, which are true hermaphrodites. Aggregation and injection chimeras are human-created organisms produced through the artificial fusion of embryos or the injection of the inner cell mass or cultured cells into blastocysts. This

chapter describes injection techniques for generating knockout mice and shows how injection chimeras can be used to study tissue-specific gene function.

Interspecies chimeras

The technologies used to generate aggregation and injection chimeras opened the way to the production of chimeras of different species, including - among others - chicken/quail, rat/mouse, and sheep/goat chimeras. Interspecies chimeras have been of great use in elucidating the process of cell migration during embryogenesis, contributing to our understanding of the nature of foetus-mother interactions. The reasons for the failure of attempts to generate, e.g., pig/human chimeras are also discussed.

Chimeras from grafting and transplantation

This chapter describes how rootstock and scion are joined to produce plant chimeras and lists the advantages of their many uses in plant breeding and production. People with transplanted organs are also chimeras. Some of their interesting features are described.

3.3. Cell-exchange or microchimeras

It was discovered during the late 1960s that a high number of mothers carry cells from their foetus(es) and vice versa. Apparently, it happens rather frequently that a mother and her foetus exchange cells. Interestingly, the “foreign” cells may live in their new “host” for decades. (Because the number of the “foreign” cells is low - as compared to “host” cells - the phenomenon is called microchimerism.) The foetus-mother cell exchange makes the isolation of foetal cells from the mother’s blood for chromosome and DNA analyses possible. Freemartins (sterile female calves that have male twins) are interesting examples of cell-exchange chimeras.

The condition in which an organism is composed of cells with different geno- and/or phenotypes is known as genetic mosaicism. Genetic mosaics are organisms with cells with different geno- and/or phenotypes. Variegation implies the simultaneous presence in an organ of cells that appear wild type and cells that display the mutant phenotype. Apparently, it is almost certain that every multicellular organism is a genetic mosaic. Many genetic mosaics are beautiful and are propagated by plant and animal breeders. Genetic mosaics are also routinely generated to study interesting biological processes. Genetic mosaicism is a basic component of tumor

formation. The study of genetic mosaics is an important tool for examining environmental mutagenesis. The book in your hands introduces the different types of genetic mosaics, covers the mechanisms of their origins, and illustrates their relevance in our lives. It also aims to introduce some beauties and rather unusual phenomena of life. The author of the book is well aware of the difficulties in reading a text that is rich in the technical terms of genetics and developmental biology. At the end of the book a glossary of terms is provided with the aim of making reading and understanding this book easier.

CHAPTER 1

TRUE GENETIC MOSAICS

1.1. Point mutations and mosaic spots

An inherent feature of DNA is that mutations emerge spontaneously - seldom, but constantly - in the course of its replication. Most mutations are of the base-pair exchange type or originate through the deletion or the addition of one or a few base pairs. Mutations also emerge as the consequence of physical, chemical, or biological effects. Among biological mutagens the so-called mobile genetic elements (transposons, jumping genes) are of great importance. While changing their positions in the genome, transposons may become inserted into genes, thus eliminating gene functions. A transposon may also become removed from a gene; an event that may well be followed by restored gene activity.

Let us consider a leaf primordium (Figure 1.1). The genes engaged in the synthesis of yellow carotene pigments are intact and function normally. One of the chlorophyll genes also functions normally and codes for the formation of chlorophyll, the green photosynthetic pigment. However, the other chlorophyll gene is non-functional. If a mutation occurs in the normal chlorophyll gene in a cell of a leaf primordium - e.g., because a transposon becomes inserted - the gene loses its function. Such a cell forms only carotenes and not chlorophyll. (Note that mutations that occur in somatic cells are called somatic mutations. Their effects are restricted only to the somatic cells, which do not contribute to the genetic contents of offspring.) Although the yellow cell does not perform photosynthesis, it will survive thanks to the support of its neighbouring green cells. It will keep on dividing. The cells that descend from it will stay together and from a yellow spot, a clone, in the midst of the green cells of the leaf (Fig. 1.1). It is important to emphasize that the cells of a clone (a mosaic spot) descend from a single founder cell.

It can occur that the transposon is removed from the chlorophyll gene of a mutant yellow cell and the function of the gene becomes restored. The formerly yellow cell and the cells descending from it will resume

chlorophyll synthesis and form a green spot among the yellow cells (Fig.1.1).

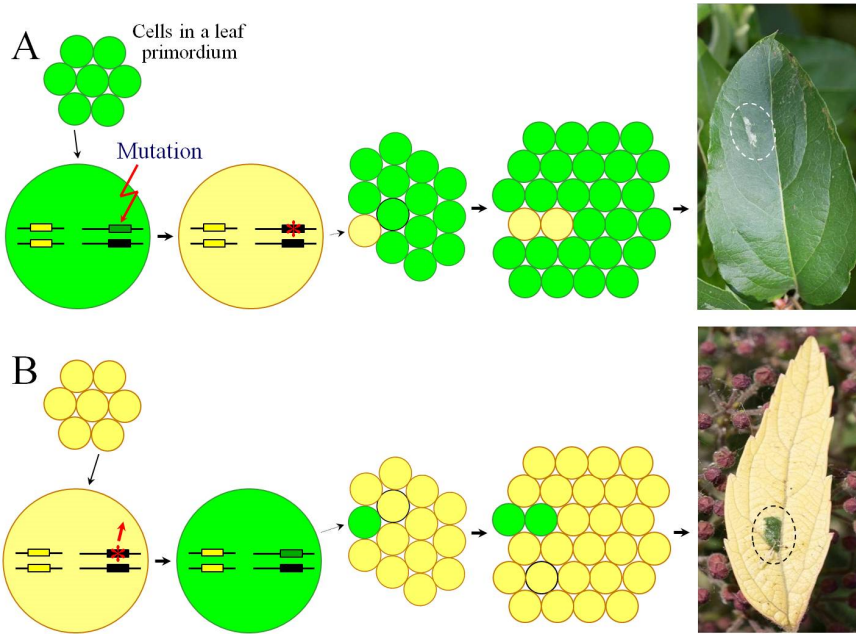

Figure 1.1. Formation of yellow/green leaf mosaicism.
(A) A yellow clone forms in the midst of green leaf cells. (B) A green spot develops surrounded by yellow cells. The yellow rectangles symbolise genes engaged in the synthesis of yellow carotene. The green rectangle symbolises the gene responsible for chlorophyll synthesis. The black rectangle stands for a non-functional chlorophyll gene. (See text for details.)

A further example of somatic mutation-related mosaicism is a so-called firemark (also known as a port-wine stain or nevus flammeus), a type of birthmark (Figure 1.2). Firemarks are discolorations of the human skin caused by capillary malformations in the skin. Firemarks are already present at birth, appearing pinkish, and deepen to dark red or purplish as the child gets older. The colour of the spot is similar to a fortified red wine from Portugal, hence its other name, port-wine stain. The affected region of skin grows in proportion to general growth. Firemarks appear most frequently on the face or the neck. However, they may develop anywhere on the surface of the body. An important feature of firemarks is that they persist throughout the lives of affected people.

Firemarks are caused by somatic mutations in a cell of the embryo, in the *GNAQ* [*Guanine nucleotide-binding protein G(q) subunit α*] gene. The *GNAQ* gene has important roles in capillary formation and functions in the skin. A product of the *GNAQ* gene, the Gαp protein is a component of a signal transduction cascade inside the cells. The firemark-causing mutation is a replacement of the G≡C base pair by an A=T base pair at position 548 in the gene. The consequence of the base pair exchange is the replacement of the 183rd amino acid: arginine (R) with glutamine (Q). The mutant R182Q-Gαp molecules are continuously active and prevent the narrowing of the capillaries. The capillaries that originate from descendants of the cell with the $G^{548}\rightarrow A$ mutation are slightly more dilated than normal, and the extra blood inside turns the skin over that region of the skin red. When inherited, the $G^{548}\rightarrow A$ mutation is dominant.

Somatic mutations in the *GNAQ* or in the *GNA11* genes have been reported to be present in cherry (senile) angiomas, which are red papules on the skin (Fig. 1.2). Cherry angiomas are harmless benign tumours that originate from an excess proliferation of blood vessels. They appear in nearly all adults over 30 and increase by a few mm in size with age. (The product of the *GNA11* gene is involved in making the Gαp protein.)

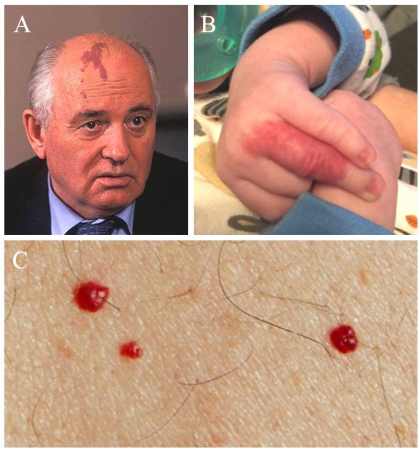

Figure 1.2. Firemarks and cherry (senile) angiomas.
(A, B) Fire spots. The capillary vessels are wider than normal over the affected region. The purplish colour originates from the blood inside the capillaries. (C) Cherry angiomas, small harmless papules composed of clusters of capillaries on the skin surface.

Salmon patches (stork bites, nevus simplex) are a harmless type of birthmark. They develop on almost every other new-born baby. Their pinkish appearance is caused by the blood in the dilated capillaries under the skin. Salmon patches appear most frequently on the neck and the face, sometimes on the forehead or the eyelids. They usually disappear within a few months. Although salmon patches seem to be inherited, their genetic basis is yet unknown. Further examples of point mutation-based mosaicism are shown in Figure 1.4.

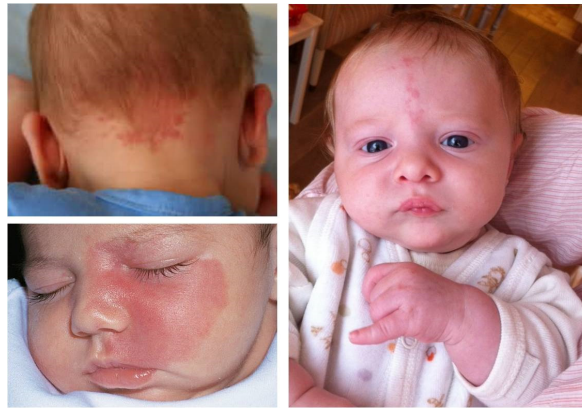

Figure 1.3. Salmon patches or stork bites.
The pinkish patches derive from the blood in the dilated capillaries. They usually fade away by about the 18th month.

Studying the inheritance of mosaic patterns in maize kernels led to the discovery of mobile genetic elements in the 1940s and was honoured with the Nobel-prize awarded to Barbara McClintock in 1983. Red/white variegation in roses and purple/white spots in beans may well be the consequences of point mutations in the colour-coding genes. Dark spot in the white wool of the lamb was caused by a back-mutation that restores the melanin synthesis in a cell during early embryogenesis. Bluish tail feathers in white pigeons, age spots, and blue spots in brown eyes may also be caused by point mutations.

Figure 1.4. Mosaic spots that might have originated due to point mutations.

Symmetric and non-symmetric spot patterns

Yellow/green mosaic plants and people with firemarks are genetic mosaics: they are composed of cells that have different geno- and phenotypes. As the mutations that lead to the formation of mosaic spots happen randomly in primordial cells, the distribution of mosaic spots is also random. When mutations occur in a young primordium composed only of a few cells, the founder cell can divide many times during the development of the organ, which leads to the formation of a large clone, though such early clones are rare. When the mutations happen in older organ primordia already composed of many cells, several small clones form: the progeny of a founder cell will divide only a few times before the completion of organ development. Furthermore, the shape of mosaic spots is irregular since the positions of affected cells are not predetermined (see Figures 1.1, 1.2, 1.3 and 1.4). In summary, there are three important characteristics of genetic mosaicism: (i) random distribution of mosaic spots, (ii) wide range in spot size, and (iii) irregular spot shape (Figure 1.5).

The above examples illustrate typical features of genetic mosaics. Organisms with regular patterns of colourful spots are rather common and possess symmetric arrangements of spots (Figure 1.5). The basis of such symmetric distribution is spatially organized gene expression activity. Although the genetic composition is identical in every cell of a developing organ or organism, the positions of the cells are different. The cells are

thus exposed to different factors that regulate gene expression and/or to different concentrations of the same factor. The formation of eyespots on the wings of, e.g., peacock butterflies depends on the types and concentrations of diffusing molecules that reach the cells in the wing primordia and induce or prevent pigment formation.

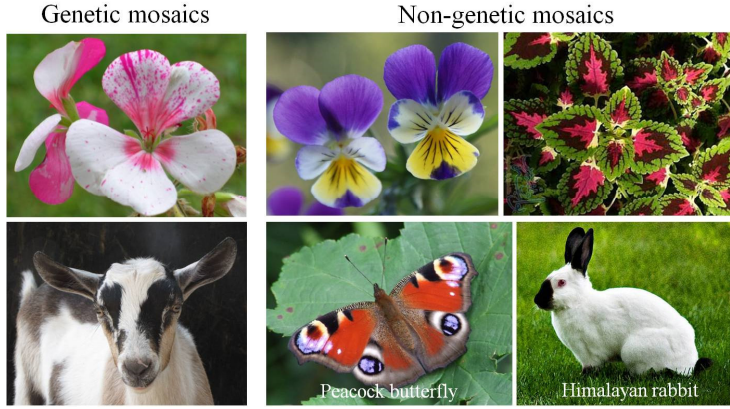

Figure 1.5. Colour patterns are non-symmetric in genetic mosaics and symmetric in non-genetic mosaics.
Eyespot formation in the Peacock butterfly is organized by two spatial coordinate systems. The first specifies the positional information of the cells in the wing primordium, while the other organizes the formation of the colour patterns. The Himalayan rabbit carries c^{ch1}, the temperature-sensitive mutant allele of the *complete colour* gene. The c^{ch1} allele encodes the formation of an enzyme that functions in the lower-temperature body regions (ears, nose, feet, and tail) and forms melanin, the black pigment. The enzyme does not function in body regions with higher temperatures, so the rest of the rabbit's fur remains white (colourless).

Point mutation and tumour formation

Yellow/green mosaic leaves, red/white spotted flowers, and mosaic animals are eye-catching. People keep and propagate them. Additionally, the mutation-based type of mosaicism also has practical applications.

Imagine someone who was born with a loss-of-function mutant allele of the *retinoblastoma* (*rb*) gene. (The *rb* gene is a member of the so-called tumour suppressor gene family. Its product, the RB protein, is present in

every cell and functions to prevent uncontrolled cell proliferation.) The functional RB protein binds to the E2F protein and prevents its activity. The E2F protein is a transcription factor involved in cell cycle regulation. (Transcription factors are DNA-binding proteins that regulate gene expression.) In the absence of the functional *rb* gene or RB protein, the E2F protein can induce uncontrolled cell proliferation, creating tumours.

When a mutation occurs - mostly during replication - in the normal (+) gene in an *rb*/+ retina cell and a new loss-of-function mutant *rb'* allele forms, one of the daughter cells becomes homozygous for the *rb* mutant allele following mitosis (Fig. 1.6). Since such a cell does not carry functional RB proteins, the E2F protein can freely function, inducing uncontrolled cell proliferation and the formation of a retinoblastoma, a malignant tumour of the retina (Figure 1.7). People with both *rb*/+ and *rb*/rb' cells are genetic mosaics. Descendants of the *rb*/*rb'* cell form a clone.

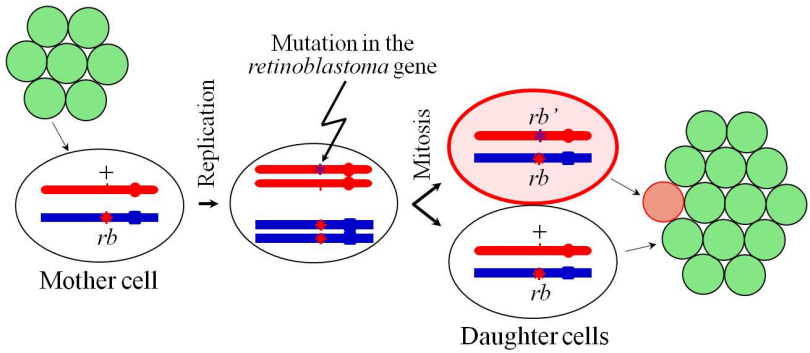

Figure 1.6. The relationship between a point mutation and tumour formation.
A mutation in the normal (+) *rb* gene leads to the formation of a new mutant allele (*rb'*). The forming *rb*/*rb'* cell is now homozygous for the *rb* mutation and may be the source of a tumour.

About 30-40% of retinoblastoma cases are familial in nature, i.e., the trait appears frequently among family members. Analyses of DNA isolated from retinoblastoma tumours have clearly revealed that many are caused by the mechanism depicted in Figure 1.6: a point mutation in the normal *rb* gene in usually one cell of the heterozygote. As will be described in a later chapter, another genetic mechanism can also lead to loss of "heterozygosity", the formation of cells homozygous for the *rb* mutation and the formation of tumours. (Although it is rare, it does happen that

mutations destroy both normal *rb* alleles in the same cell lineage at different points in time. Such cells without normal *rb* genes may also become the sources of retinoblastomas.)

DNA-based studies have revealed that point mutations in normal alleles in heterozygotes - as depicted in Fig. 1.6. - are among the events that may lead to colon cancer and breast cancer (Fig. 1.7). In colon cancer the point mutations frequently eliminate the function of the *APC* (*adenomatous polyposis coli*) gene. (A product of the *APC* gene, the APC protein, is involved in the regulation of cell proliferation in association with a few other types of proteins.) Mutations in a normal *BRCA* (*breast cancer*) tumour-suppressor gene may lead to the formation of breast cancer. (BRCA proteins, products of the *BRCA* genes, are engaged in DNA repair. Failure of DNA repair frequently results in mutations.)

Understanding the effect of mutations on *retinoblastoma*, *APC*, *BRCA*, and many other genes reveals the role of mutations in cancer formation. Furthermore, people heterozygous for, e.g., a mutant *rb* allele can be identified and warned of the lurking threat of cancer.

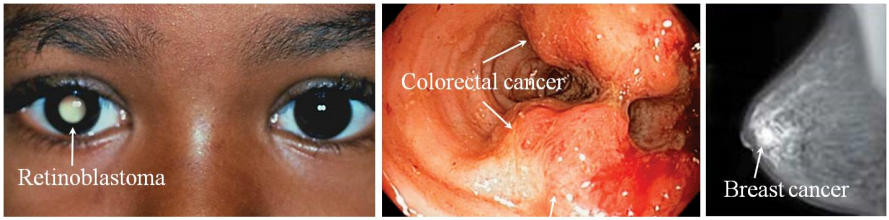

Figure 1.7. Point mutations may lead to cancer formation.
A retinoblastoma tumour behind the pupil makes it appear white. Many colorectal tumours can be recognized and removed before they become malignant. Mammography helps identify breast tumours early.

Mosaics and mutagenicity testing

Since point mutations can lead to the formation of genetic mosaics, genetic mosaics are effective tools for detecting and measuring the mutagenic activity (mutation-inducing abilities) of physical, chemical, or biological agents. This idea was implemented in the SMART (somatic mutation and recombination test) procedure. In this procedure, Drosophila larvae are exposed to potentially mutagenic agents, and the wings of the developing adults are analysed to discover whether the frequency of mosaic spots

increases following the treatment. This research is vital because every mutagen is also a carcinogen (a cancer-inducing agent), and cancer is responsible for 13% of human deaths. Remarkably, most agents that possess mutagenic activities in Drosophila are also mutagenic in humans. Of course, it is a lot more convenient to use model organisms (like Drosophila) to analyse mutagenicity than mice or human cells.

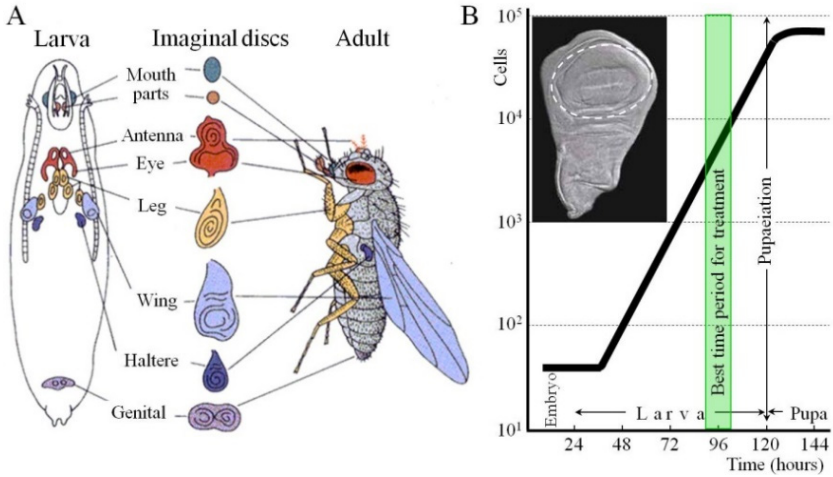

Figure 1.8. Imaginal discs and features of Drosophila wing development. (A) Much of the adult epidermis develops in imaginal discs during larval life. Imaginal discs are composed of diploid cells that keep dividing inside the larva. Following pupariation, the discs take their final positions and produce the characteristic chitin structures of the adult. (B) A wing disc forms from about 30-50 cells during embryogenesis. Since its cells divide 10-11 times during the larval stage, the number of cells increases exponentially to about 50 thousand in 100 hours (one cycle lasts about ten hours). Cell division ceases after pupariation. A wing forms from the area in the circle in the insert picture. The most advantageous age for larvae to be treated for the study described above is about 96 hours: at this stage there are several thousand cells in an imaginal disc, which will divide 2-3 times before the cessation of mitoses, and thus their descendants can form clones composed of two to four cells.

Let us consider a cell in a wing primordium of a Drosophila larva (Fig. 1.8). The larva is heterozygous for the *mwh* (*multiple wing hairs*) recessive marker mutation (*mwh*/+). (Normally every cell of a Drosophila wing

produces one trichome, or hair). A cell homozygous for the *mwh* marker mutation produces 2-7 trichomes instead of the usual one. When a point mutation occurs in the normal (+) *mwh* gene in a wing disc cell and a new mutant allele (*mwh'*) forms, the cell becomes homozygous for *mwh* (Fig. 1.9). Descendants of such a founder cell stay together and form an *mwh* clone in the midst of normal cells in the developing wing. A treatment is considered mutagenic when it induces *mwh* clones with a significantly higher frequency than seen in the control. This principle is illustrated by the data summarized in Table 1.1: each of the studied chemical compounds induced *mwh* clones at higher frequencies than in the control. It is important to note that - as shown in the control (Table 1.1) - *mwh* clones do form spontaneously, although rarely.

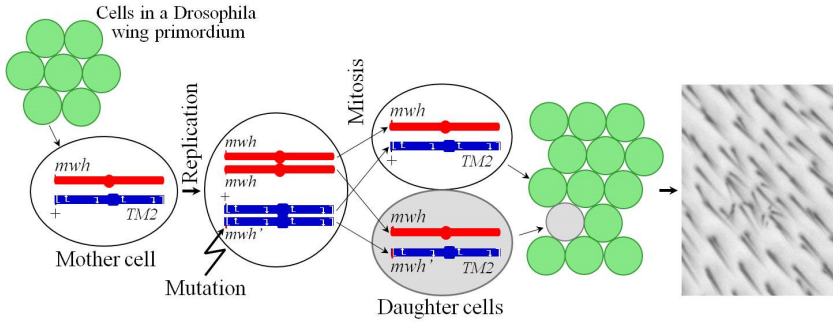

Figure 1.9. Point mutation and the formation of a mosaic spot.
Following the induction of a mutation in the normal (+) *mwh* gene in an *mwh*/+ Drosophila wing disc cell, one of the daughter cells becomes *mwh* homozygous (*mwh*/*mwh'*). Its descendant cells will produce 2-7 trichomes each instead of the usual one, forming a clone in the emerging wing. (There are four *mwh*/*mwh'* cells in the clone in the figure.) The symbols used are as follows: *mwh'* stands for the newly induced *mwh* mutant allele. *TM2* stands for a so-called balancer chromosome that carries the normal (+) *mwh* gene and three inversions. *TM2* ensures that the *mwh* clones develop only following the induction of point mutations and not due to mitotic recombinations, as described in the next chapter.

Table 1.1. Detection of mutagenic activity and its strength based on *mwh* clones on Drosophila wings

Treatment	Wing *N*	*mwh* clone *n*	Clone frequency *n*/*N*	Average clone size (cells/clone) *m*	Frequency of clone induction $f = n\,2m/NC$
Control	116	9	0.08	2.7±1.6	1.4×10^{-5}
Ethyl methanesulfonate (EMS; 1 mg/ml, 4 hours)	101	26	0.26*	2.4±2.1	4.1×10^{-5}
Hexamethylphosphoramide (HMPA) 1% in the food, 3-4 days	40	30	0.30*	2.9±2.0	14.5×10^{-5}
N-Nitrosomorpholine 0.64 mmol/kg in the food, 3-4 days	14	9	0.64*	3.6±2.6	15.4×10^{-5}
N-Methyl-N-nitroso-p-toluenesulfonamide 4.67 mmol/kg in the food, 3-4 days	100	30	0.75*	2.5±1.5	5.0×10^{-5}

* Significantly different from the control ($P<0.001$)
- The carcinogenic effect of nitroso compounds has been proven.
- The nitroso compounds were mixed into standard Drosophila food and the larvae were fed it for 3-4 days.
- HMPA (hexamethylphosphoramide) is a well-known carcinogen. It is a polar aprotic solvent and an additive in organic synthesis.
- In the fifth column average±standard deviation values are listed.

Sources of the data: Mutation Research **144**, 177, 1985 and Mutation Research **173**, 197, 1986.

This test is most effective when many cells are exposed to the mutagen, which is to say that relatively older larvae are treated (Fig. 1.8). In such larvae a wing disc already contains thousands of cells, and there is still time for a few rounds of cell division so that the cells can transmit their *mwh*/*mwh'* genotype to the descending cells before cell division ceases shortly after pupariation. The best age for the exposure of larvae to the tested agent is 96 hours after the initiation of embryogenesis, when roughly three thousand cells compose a wing disc (Fig. 1.8). A study involving about a hundred wings means the exposure of hundreds of thousands of cells to the studied agent. As there are two or three cell divisions left after 96 hours of development, the *mwh*/*mwh'* clones will

contain 2-4 cells and can be recognized easily. In the procedure the wings are detached from the flies and are prepared for analysis (Fig. 1.10). The wings are then screened in a compound microscope. The number, size, and position of the *mwh* clones are recorded.

Figure 1.10. Wings prepared for analysis and picture of a Drosophila wing. A wing is 0.4-0.5 mm, a trichome is about 10 µm long. There are about 30 thousand cells in a Drosophila wing.

Dominant mutations and mosaicism

The previous chapter dealt with the recessive, loss-of-function type of mutations. Most newly arising mutations are the loss-of-function type and cause partial or complete elimination of gene functions. The vast majority of loss-of-function mutations are recessive, i.e., their phenotypes are manifested only in homo- or in hemizygous conditions. (In hemizygotes one of the alleles is missing, either because the gene was lost or is present in only one copy, like X-linked genes in XY males.)

However, a number of mutations are dominant. Table 1.2 provides a classification of the types of mutations. Interestingly, some loss-of-function mutations are dominant, i.e., the loss of one of the two normal gene copies leads to malfunction, which implies that a single functional gene copy is insufficient to sustain normal cell functions. This condition is known as haploinsufficiency and is usually associated with characteristic mutant phenotypes. In fact, haploinsufficiency is a major cause of dominantly inherited diseases. It is estimated that there are a little over one thousand haploinsufficient genes in humans (among the 20,412 protein-encoding genes). Naturally, the loss of a haploinsufficient gene in a cell of an organ primordium may well lead to mosaicism. We mention three examples here.

Table 1.2. A classification of mutations

Nature	Type	Genotype	Condition	Phenotype when an extra normal (+) gene is present
Recessive	Loss-of-function	*u*/*u* or *u*/–	Characteristic mutant phenotype	*u*/*u*/+ Becomes normal
	Dose-sensitive	*u*/+ or –/+	Haploinsufficient	*u*/+/+ or –/+/+ Becomes normal
		+/+/+	Triplo-abnormal	+/+/+/+ Becomes worse
Dominant		*H*/+	Hypermorph	*H*/+/+ Becomes slightly worse
	Gain-of-function	*D*/+	Dominant negative	*D*/+/+ Slightly improves
		N/+	Neomorph	*N*/+/+ Does not change

+ stands for the normal gene, *u* for a loss-of-function mutant allele, and – for a deficiency that removes the normal gene. *H* symbolises a so-called hypermorph, *D* a dominant negative, and *N* a neomorph mutant allele. Hypermorph mutations cause an increase in normal gene function as the consequence of an increase in mRNA or protein expression, or constitutive activity of the encoded protein.

The loss of the *Minute (4)* gene - along with one of the two 4th chromosomes - during Drosophila development leads to a haplo-4 mosaic possessing haploinsufficiency (Fig. 1.11). The *Minute (4)* gene encodes one of the ribosomal proteins. Reducing its concentration by 50% results in the formation of bristles that are short, thin, and pale (Fig. 1.11). In humans, the absence of one of the two *PRPF31* (*pre-mRNA processing factor 31 homolog*) genes leads to retinitis pigmentosa, progressive degeneration of the photoreceptor cells in the retina, severe visual impairment, and blindness (Fig. 1.11). The *PRPF31* gene encodes the formation of a protein engaged in pre-mRNA splicing. Apparently, a single copy of the *PRPF31* gene is insufficient to supply sufficient amounts of the hPRPF31 protein and, therefore, the loss of a copy of the *PRPF31* gene reveals that this mutation exhibits dominance in heredity. Another example of haploinsufficiency in humans is elliptocytosis, the formation of elliptical red blood cells instead of

donut-shaped ones (Fig. 1.11). In two-thirds of the cases of hereditary elliptocytosis, one of the copies of the *SPTA1* (*spectrin alpha, erythrocytic 1*) gene is missing. The *SPTA1* gene encodes α1-spectrin, a component of the cytoskeleton. The decrease of the α1-spectrin concentration by 50% leads to an unstable cytoskeleton and abnormal red blood cell membrane behaviour. When passing through the capillaries, red blood cells become elliptical. After leaving the capillaries, normal red blood cells regain their normal shape; however, those with an abnormal cytoskeleton remain elliptical. Such cells are then removed from circulation in the spleen and thus live a lot shorter lives than the average red blood cell, which has a lifespan of 120 days. Early removal of the erythrocytes leads to hemolytic anaemia.

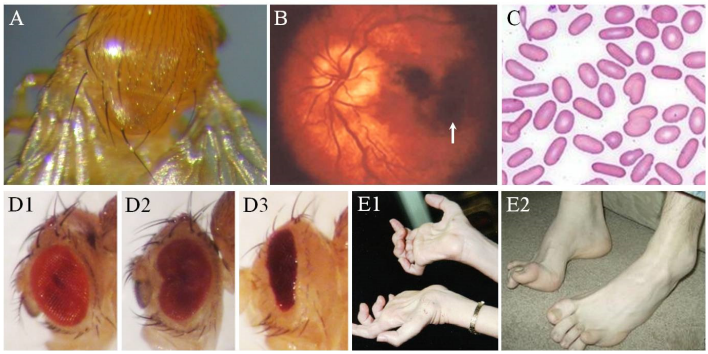

Figure 1.11. Haploinsufficiency and the triplo-abnormal condition.
(A) The thorax of a Haplo-4 Drosophila mosaic. While the bristles are normal on the left side, they are short, pale, and thin (Minute) on the right side. (B) The loss of one of the *PRPF31* genes in a retina primordium cell caused sectorial retinitis pigmentosa (↑), the degeneration of the photoreceptor cells. The condition is related to haploinsufficiency of the *PRPF31* gene. (C) Haploinsufficiency of the *SPTA1* gene - which encodes the formation of α1-spectrin - in some blood-producing stem cells leads to mosaic elliptocytosis, the presence of elliptical red blood cells among normal disc shaped red blood cells. (D1) Eyes of most Drosophila females (+/+) are round and contain about 800 regularly arranged ommatidia. (D2) An extra copy of the *Bar* gene (+/+/+) leads to a triplo-abnormal condition: eyes with about 360 irregularly arranged ommatidia. (D3) Two extra copies of the *Bar* gene results in ultra-bar eyes with some 70 ommatidia. (E1 and E2) The triplo-abnormal condition of the *PMP22* gene brings about the development of Charcot-Marie-Tooth disease, an inherited disorder of the peripheral nervous system: progressive loss of touch sensation, muscle degeneration, and abnormal hands and feet.

There are genes which, when present in three normal copies instead of the usual two, disturb cell functions, leading to a so-called triplo-abnormal condition (Fig. 1.11). When the Drosophila *Bar* gene - which encodes a transcription factor - is present in three copies instead of the usual two, the eyes become kidney-shaped with disorganized ommatidia, which are units of the compound eyes (Fig. 1.11). Four normal *Bar* genes cause bar-shaped eyes. In humans an extra copy of the *PMP22* gene - which encodes peripheral myelin protein 22, a major component of the peripheral neuron myelin sheath - results in Charcot-Marie-Tooth disease: progressive loss of the peripheral nervous system, followed by the loss of touch sensation, muscle degeneration, and the distortion of hands and feet. On the other hand, haploinsufficiency of the *PMP22* gene brings about peripheral neuropathy, which manifests as abnormal sensation in the arms and legs, impaired muscle movement, and paralysis. More examples of triplo-abnormal genes are listed in the chapter on chromosomal mosaics.

What is the basis of haploinsufficiency and triplo-abnormality? In most genes the concentration of the gene product does not depend on the number of encoding genes (whether one, two, three, or more). However, there are genes that are required in two copies for normal cell functions: the concentration of the encoded gene products depends on the number of copies of the gene. The absence of one copy of the gene leads to haploinsufficiency, while an extra copy leads to the triplo-abnormal condition. In such genes the biochemical balance of the cells becomes upset, which leads to different types of abnormalities. Of the 13,733 protein-encoding Drosophila genes, only 57 are of the haploinsufficient type. Most of the 57 genes encode the formation of ribosomal proteins. Although the number of triplo-abnormal genes is not known, there are many more than haploinsufficient ones. It appears that cells better tolerate the gain than the loss of genes.

Loss-of-function mutations arise 2-3 thousand times more frequently than gain-of-function types. Most gain-of-function mutant alleles are dominant and encode the formation of gene products that have adverse effects on cells and organisms. There are three types of gain-of-function mutations (Table 1.2). The so-called hypermorphs produce excess amounts of the normal gene product. Dominant negative mutant alleles encode the formation of mutant gene products that interfere with the functions of the normal gene products, implying that the two types of gene products participate in the same process. (The phenotypes associated with dominant negative mutations are therefore pretty much the same as the phenotypes of the loss-of-function recessive mutants.) Products of the neomorph